20030310138

ORAL PHARMACODYNAMIC BIOAVAILABILITY OF SIX p-AMINOPHENONE DERIVATIVES

Larry D. Brown, Thomas G. Brewer, Douglas R. Flanagan*, Jurgen D. von Bredow, Ralf P. Brueckner, and Dr. Robert R. Engle.

Division of Experimental Therapeutics, Walter Reed Army Institute of Research (WRAIR), Washington, DC 20307-5100;
*School of Pharmacy, University of Iowa, Iowa City, Iowa 52242.

ABSTRACT

A number of potential biochemical approaches exist for protection against and treatment of cyanide poisoning. The methemoglobin protective approach appears to be the most promising and practical. Adequate protection against the lethal effects of cyanide intoxication depends upon a sufficient amount of available methemoglobin to bind and remove the toxic cyanide ion from the circulation. The intravenous administration of solutions of direct and indirect methemoglobin formers WR000302 (p-aminopropiophenone, PAPP), WR269,410 (p-aminoheptanophenone, PAHP), WR258,948 (p-aminooctanophenone, PAOP) and their respective N-hydroxy derivatives (WR270,101, WR272,677 and WR271,159) to unanesthetized beagle dogs leads to a rapid increase in whole blood methemoglobin. In the absence of a specific analytical method for the accurate analysis of each of these six compounds, the corresponding whole blood methemoglobin induced by each of these compounds were monitored by the Radiometer OSM-3 hemoximeter immediately following blood sampling to construct a pharmacodynamic profile of methemoglobin vs time. When these same animals were given oral gavage doses of either a solution or suspension of the same propiophenone derivative, a rapid and consistent increase in methemoglobin levels was also observed which may be used as a measure of the rate and extent of absorption of the compound into the whole blood

Best Available Copy

COMPONENT PART NOTICE

THIS PAPER IS A COMPONENT PART OF THE FOLLOWING COMPILATION REPORT:

TITLE: Proceedings of the Medical Defense Bioscience Review (1993) in Baltimore, Maryland on 10-13 May 1993. Volume 2.

TO ORDER THE COMPLETE COMPILATION REPORT, USE AD-A275 668.

THE COMPONENT PART IS PROVIDED HERE TO ALLOW USERS ACCESS TO INDIVIDUALLY AUTHORED SECTIONS OF PROCEEDING, ANNALS, SYMPOSIA, ETC. HOWEVER, THE COMPONENT SHOULD BE CONSIDERED WITHIN THE CONTEXT OF THE OVERALL COMPILATION REPORT AND NOT AS A STAND-ALONE TECHNICAL REPORT.

THE FOLLOWING COMPONENT PART NUMBERS COMPRISE THE COMPILATION REPORT:

AD#: AD-P008 795	AD#:
AD#: thru	AD#:
AD#: AD-P008 852	AD#:

Accesion For	
NTIS CRA&I	☑
DTIC TAB	☐
Unannounced	☐
Justification	
By	
Distribution /	
Availability Codes	
Dist	Avail and / or Special
A-1	

circulation. The pharmacodynamic profiles of the intravenous dose can be compared with the oral gavage dose of the two formulations (solution and suspension) to obtain an estimate of pharmacodynamic oral bioavailability. This estimate can be used to select the compound and formulation which provides the necessary duration of methemoglobin sufficient to protect against possible exposure to cyanide.

INTRODUCTION:

HCN is a chemical warfare threat agent. Cyanide (CN^-) is an intracellular poison that binds to cytochrome oxidase, thereby disrupting the electron transport system. This interruption of cellular respiration blocks ATP production and rapidly leads to cell death. Hydrogen cyanide (HCN) is highly volatile and when inhaled by man in high concentrations causes dizziness and confusion within seconds. Respiratory arrest may occur in less than one minute. MOPP gear provides soldier protection if donned immediately and gas mask filters remain functional.

Current cyanide treatment requires expedient intravenous administration of approved drugs (sodium nitrite and sodium thiosulfate) which, on the battlefield, is impractical. Therefore, prevention of cyanide-induced casualties by prophylactic measures is essential. Drugs that induce methemoglobinemia have been recognized as effective cyanide antidotes for years. Methemoglobin (MHb) contains an oxidized ($Fe3+$) form of heme iron that has a higher CN- binding affinity than does cytochrome oxidase. Preferential binding of CN- to MHb to form cyanomethemoglobin removes it from the cytochrome oxidase active site and restores normal cell respiration.

A safe, well tolerated, and effective prophylactic for soldiers who may be exposed to cyanide is needed. The ideal treatment should provide adequate protection (against at least 2 LD_{50}s of cyanide), be easily administered (preferably orally) at daily or less frequent intervals, and not interfere with soldiers' performance.

Methemoglobinemia is known to protect mammals against lethal doses of cyanide. It is necessary to evaluate the kinetics of methemoglobinemia induced by candidate drugs to determine whether methemoglobin formation and elimination rates are rapid enough as well as predictable and stable enough to be used in a long term dosing regimen in soldiers. Bioavailability is one of the most important parameters for drug development candidate advancement decision making. Formulation modifications can enhance the bioavailability of orally delivered drugs but gains in this area are limited, therefore candidates with the best inherent absorption must be identified. Bioavailability is defined by the FDA as both the extent (relative amount) of drug from an administered dosage form which enters the systemic circulation and the rate at which the drug appears in the blood stream and presumably at the site of dynamic action. Three types of bioavailability (absolute, relative, relative optimal bioavailability) are described in the literature. Absolute bioavailability (F) is calculated by the following formula: F = AUCpo/AUCiv and is the fraction of drug absorbed by any nonvascular route in relation to the intravenous dose which is assumed to be 100%. Absolute bioavailability have values which range from 0 < F < 1. A drug with an F value of 1 has 'perfect' or 100% oral bioavailability equivalent to direct intravenous infusion.

Six p-aminophenones were studied in the male beagle dog to determine the single dose time-concentration curves for methemoglobin formation after intravenous and stomach gavage (POG) routes. Bioavailability (F) was calculated for both solution and suspension formulations of these six compounds to allow advancement rank ordering based upon this data in concert with other available information and for improvements in formulation, if needed, for future planned pharmacology studies.

This study evaluated the utility of using 'effect kinetics' or the pharmacokinetics (PK) of a dynamic effect, i.e., methemoglobin (MHb) formation as an estimate for drug or metabolite bioavailability and as a surrogate for drug time-concentration data.

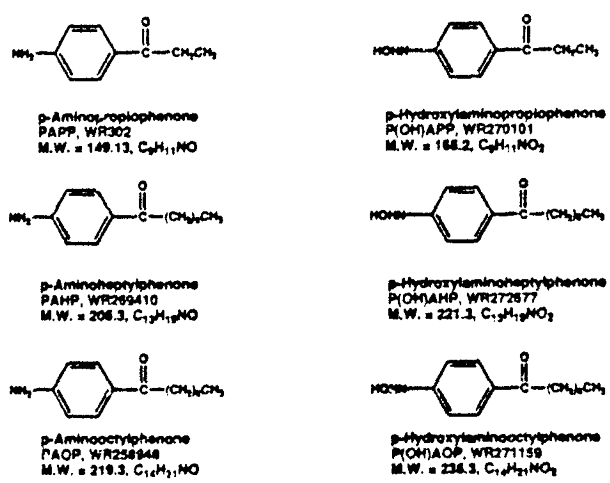
MATERIALS & METHODS:

Single intravenous and oral gavage formulations of six candidate p-aminophenones were administered to beagle dogs. This effort required intensive blood sampling and time-sensitive analysis of large numbers of samples. We routinely conducted a study using 4 animals each day with immediate measurement of MHb values. This manuscript is based upon data collected from 72 dog experiments and approximately 1,450 blood samples. Information was collected from each animal during three separate experiments (intravenous, gavage solution, gavage suspension) using a crossover design.

CHEMICAL COMPOUNDS:

PAPP, PAOP, PAHP (indirect methemoglobin formers) and the respective N-OHs (direct formers) for these three aminophenones were obtained from GMP certified inventory stock which had been synthesized under Army contract for the WRAIR Experimental Therapeutics Repository (over 275K distinct chemical compounds).

Phenones



SELECTION and CARE OF MODEL:

The dog model is the most similar to humans and the most predictive of methemoglobin forming as well as clearing activity in humans. Rodents are poor MHb formers. Methemoglobin reductase activity is substantially higher in mice than in humans. The Beagle dog has been used in previous pharmacokinetic/ pharmacodynamic studies of methemoglobin with excellent success and all available data to this point for MHb formation with 8-aminoquinolines derives from the dog model. Ruminants form methemoglobin faster and more extensively than other large mammals although the MHb-reductase activity of adult dogs and sheep is similar. The presence of the rumen substantially alters the absorptive physiology of sheep so they cannot be used as a model for orally dosed drugs. Swine have dissimilar methemoglobin reductase levels to those in humans.

A total of 28 domestic born purpose bred young adult male HRA Strain Beagle dogs (*Canis familiaris*) obtained from Hazelton Research Products weighing between 7 and 14 kg were used in these studies. Animals were one-two years of age, identified by ear tatoo, and housed at the WRAIR. Animals were cared for under veterinary supervision in accordance with the principles in the Guide for the Care and Use ofLaboratory Animals (Department of Health, Education and Welfare Publication, NIH 86-23).

DOSING PROCEDURES:

Oral and intravenous dosing formulations were prepared fresh in either a PEG 200 solution or 2% carboxymethylcellulose (CMC) suspension. For oral gavage dosing, a Surgilube® coated 16 French Salem sump gavage tube was passed per os into the stomach in the consious animal. The dose was administered followed by a flush with 10 ml of tapwater. Dogs were observed closely for 4 hours after each dose for emesis. Vomiting was not observed in the 46 gavaged dogs. Dogs were held NPO (for food only) for 12 hours before each dose and 4 hours afterwards (food and water). For intravenous dosing, the drug was prepared in filter sterilized polyethylene glycol (PEG) 200 (Sigma Chemical Co). Depending upon the solubility of each drug and the animal weight, intravenous volumes of 5 to 12 milliliters of PEG solution containing drug were infused over a three minute period into an indwelling intravenous catheter using a Harvard Pump, followed by a flush of 10 milliliters of heparinized saline. Dose levels studied included: 0.4 mg/Kg for PAPP, 6.0 mg/Kg for PAHP, 15.0 mg/Kg for PAOP and isomolar doses (0.75,1.0 and 1.06 mg/Kg, respectively) for the N-hydroxy derivatives or putative metabolites of these three parent phenones.

BLOOD SAMPLING and ANALYTICAL METHODS:

Samples were normally collected from the contralateral catheter to the one used for infusion. Venous whole blood samples were collected in heparinized syringes. 0.5 ml of 'waste' blood was removed from the catheter prior to collection of the measured sample to remove heparinized saline flush solution diluted blood.

Samples were immediately measured for total hemoglobin (gms %), HbO2 (%), HbCO (%), MHb (%) and O2ct (vol%) using the OSM3 Co-Oximeter (Radiometer America, Copenhagen) and then placed on wet ice until they could be spun down for plasma collection and freezing (generally within 30 mins). Whole blood and plasma samples for drug concentration determinations were frozen at -20°C and stored for future analysis when validated methods become available.

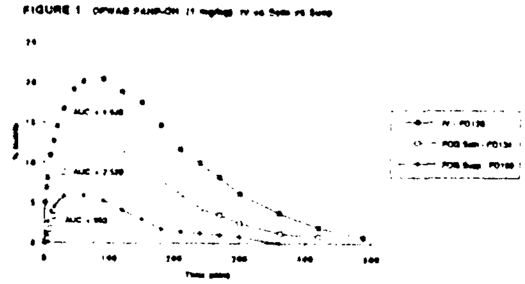
DATA and STATISTICAL ANALYSIS:

Methemoglobin time-concentration area-under-the-curve (AUC) determinations were made using the 'trapezoidal rule.' Non-linear non-compartmental 'least squares' regression analysis was performed on MHb data from three separate experiments (IV, gavage solution, gavage suspension) on each dog using the Microsoft EXCEL™ LINEST function to obtain AUCs, %AUC, MHbmax, Tmax, disappearance rate, disappearance constant, and standard statistics (s_{ey}, F, df, ss_{reg}, ss_{resid}, slope, y-intercept). The oral gavage AUCo-∞ at a given dose of each drug was divided by the intravenous AUCo-∞ at the same dose (mg/Kg) for each animal to calculate MHb bioavailability (F).

RESULTS:

Data from three separate experiments on dog DFWAB dosed with PAHP-OH are graphically represented as three methemoglobin time-concentration curves in **Figure 1**. AUCo-∞ data from these three experiments were entered into the appropriate formulas (F1 = AUCpogsoln/AUCiv; F2 = AUCpogsusp/AUCiv) for generating two separate bioavailability estimates for drug in each animal.

FIGURE 1



Summary data for 26 dog experiments (20 individual animals; 6 were used twice) which received intravenous and gavage solutions and 20 dogs which also received gavage suspensions are offered below in **Table 1**. Group mean bioavailability values (F1 for solution; F2 for suspension) for PAPP, PAOP, PAHP-OH, PAOP-OH, PAPP-OH (N=4) and PAHP (N=6), sample standard deviations and percent coefficient of variation (CV = group SD/group mean) were calculated.

TABLE 1. Group Mean Bioavailability (F) ± SD (%CV)

Candidate Drug	No. Male Beagle Dogs	F1 = AUCpogsoln/AUCiv	F2 = AUCpogsusp/AUCiv
PAHP 6 mg/Kg	(n=6)		
(7 carbon sidechain)	Mean Value ± SD (CV%)	0.4452 ± 0.1634 (36)	0.3939 ± 0.1513 (38)
PAPP 0.4 mg/Kg	(n=4)		
(3 carbon sidechain)	Mean Value ± SD (CV%)	0.5209 ± 0.3017 (58)	0.3759 ± 0.0837 (22)
PAOP 15 mg/Kg	(n=4)		
(8 carbon sidechain)	Mean Value ± SD (CV%)	0.0839 ± 0.0539 (59)	Not Conducted
PAHP-OH 1 mg/Kg	(n=4)		
(N-Hydroxy PAHP)	Mean Value ± SD (CV%)	0.6219 ± 0.1017 (16)	0.1443 ± 0.0595 (41)
PAPP-OH 0.75 mg/Kg	(n=4)		
(N-Hydroxy PAPP)	Mean Value ± SD (CV%)	0.5672 ± 0.1615 (28)	0.0823 ± 0.0264 (32)
PAOP-OH 1.06 mg/Kg	(n=4)		
(N-Hydroxy PAOP)	Mean Value ± SD (CV%)	0.3057 ± 0.0295 (9.6)	0.2588 ± 0.0642 (25)
Footnotes:	SD = Sample SD	AUCpogsoln = AUCgavage PEG 200 solution	
	AUC = AUC0-∞	AUCpogsusp = AUCgavage 2% CMC suspension	
	CV = Coefficient of Variation		

The six phenone drug candidates were then rank ordered based upon oral bioavailability for solution (F1) and suspension (F2) formulations (**Table 2**).

TABLE 2. Phenone Rank Order

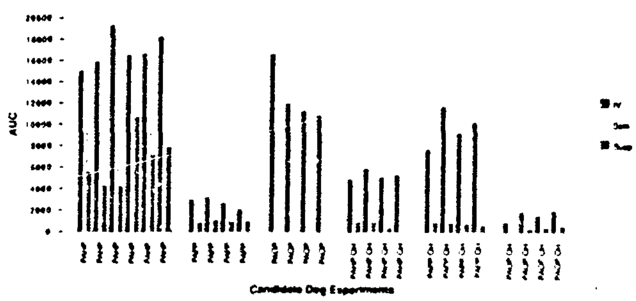
Candidate Rank Order	Soln Absolute Bioavail (F1)	Susp Absolute Bioavail (F2)	Susp Bioavail Homogeneity*
1 Best	PAHP-OH	PAHP	PAPP
2	PAPP-OH	PAPP	PAOP-OH
3	PAPP	PAOP-OH	PAPP-OH
4	PAHP	PAHP-OH	PAHP
5	PAOP-OH	PAPP-OH	PAHP-OH
6	PAOP	PAOP Not Conducted	PAOP Not Conducted

* Consistency or smallest coefficient of variation (CV)

DISCUSSION:

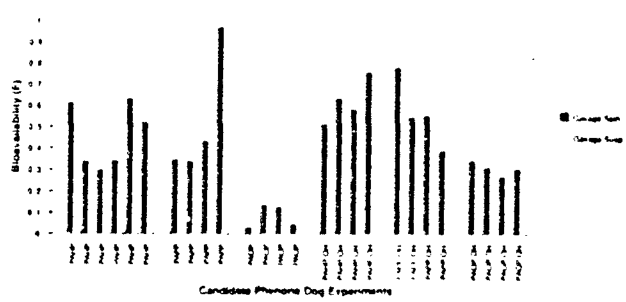
As expected, intravenous dosing resulted in the highest MHb levels (peak or Tmax) and the largest AUCs (F = 1.0), followed by gavage solutions and suspensions in that order (**Figure 2**). Gavage suspensions of PAOP were not attempted due to the very low levels of MHb observed with the PAOP gavage solution.

FIG 2. Candidate Phenone Methemoglobin Time-Concentration Areas Under the Curve (AUC) By Trapezoidal Rule



Consistent oral bioavailability is an important consideration in development of any pharmaceutical and was best for PAPP (**Figure 3**). The suspension is a special case of the aqueous solution which provides data on whether the drug can be delivered in tablet form. Based upon the evident absorption of PAPP and PAHP from these gavage suspension trials, an effective tablet formulation of these candidates would be possible.

FIG 3 Candidate Phenone Bioavailability (F) Gavage Soln vs Gavage Susp (Intravenous F = 1 For All Expts)



Rank ordering of candidates depended heavily upon the type of formulation. For instance, the 'lead candidate' (PAHP-OH) from the solution experiments changed to PAHP after the suspension experiments (**Table 2**). Additional information for final drug candidate selection will depend upon the stability of each of the compounds and the aqueous solubility over a range of pHs associated with the gastrointestinal tract (e.g., pH-water solubility profile).

These data may be used to support recommendations for rational selection of a suitable candidate drug for further development as an anti-cyanide prophylactic treatment. In addition to this bioavailability data, candidate advancement or transition and selection will depend upon other factors such as physiochemistry (stability, solubility), genetic and general mammalian toxicology outcomes, success of analytical chemistry method development, metabolism (ADME), PK/PD [duration of drug levels or dynamic effect (t1/2s), potency (Tmax), clearance (CL), Vd, Ke, AUC], dose-response data, and additional formulation considerations.

CONCLUSIONS:

• We have applied 'effect kinetics' or pharmacodynamic profiles of drug effects to estimate drug bioavailability and aid initial selection of candidate drugs in the development process. A precedent was set for this approach in prior Army research on pyridostigmine, a prophylactic pre-exposure drug for protection against nerve agent poisoning. Kinetics of that effect, i.e., reversable anticholinesterase (AcHE) enzyme inhibition, was used to rank order candidates in that project in lieu of drug level kinetics.

• Based upon the use of this concept, we have rank ordered six candidate cyanide pretreatment compounds. Drug pharmacokinetics will eventually have to be determined but only on the 'lead compounds' and not for all six candidates.

• On an absolute bioavailability basis, the N-hydroxy derivatives of PAPP and PAHP are the best candidates, however, from the practical requirement for delivery by oral tablet formulation the parents of these two compounds (i.e., PAPP and PAHP) will be the superior alternatives.

REFERENCES:

• Ritschel WA. Handbook of Basic Pharmacokinetics. Hamilton, IL: Drug Intelligence Publications, Inc., 1986.

• Rowland M, Tozer TN. Clinical Pharmacokinetics: Concepts and Applications. Philadelphia, PA: Lea & Febiger, 1989.

• Ford B. Methemoglobin inducers In: Calabrese, E.J. Principles of Animal Extrapolation. New York: John Wiley and Sons, 1983.

• Gibaldi M, Perrier D. Pharmacokinetics. New York: Marcel Dekker, Inc., 1982.

• Ballantyne B, Marrs TC (Eds). Clinical and Experimental Toxicology of Cyanides. Bristol, England: Wright Publishing, 1987.

• Kiese M. Methemoglobin. Boca Raton, FL: CRC Press, 1974.